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March 06, 2003

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Cryopreservation of large volume of semen permits whole ejaculate and double freezing of bull sperm

Genetic breeding in the field of dairy cattle includes freezing of sperm collected from young bulls for 4-5 years. In this period the young bull is evaluate by the milk production of his 70-100 daughters. The sperm submitted to artificial insemination (AI) is preserve in liquid nitrogen (LN) in 0.01 cc to 0.05 cc straws.

The bank in the Israeli AI centers preserve 24,000-30,000 straws for each bull.

The expanses for each bull for 4 years are:

| 1 ton LN (\$1,500) per 24K straws X 4years | \$6,000 |
|--|-----------|
| 60 ejaculate needs 60 days of work (three persons 180X 75) | \$13,500 |
| Food and maintains per bulls (in two years) | \$ 3,200 |
| 6 liter freezing extender | \$ 500 |
| 24,000 straws | \$ 2,400 |
| Total: | \$ 25,600 |

Typically only one bull from 14 tested is selected every year for insemination in the dairy herds, which mean that 92% of the straws are discarded without being used.

We suggest here freezing of whole ejaculate in a single test tube of 15ml instead of in 600 straws. After the 5 years of progeny testing, the tubes of the best bulls (8%) can be thawed and refrozen in conventional straws of 0.01 cc to 0.05 cc while the rest of the tubes are discarded. This practice reduces expenses as follows:

| 400 liter LN (\$600) per 60 test tube per year X 4years | \$2,400 |
|---|-----------|
| 60 ejaculates needs 60 days of work (2 person 120X 75) | \$9,000 |
| Food and maintains per bulls (in two years) | \$3,200 |
| 1 liter freezing extender | \$100 |
| 60 test tubes | \$6 |
| Total: | \$ 14,706 |

The practicality of freezing of whole ejaculates of bull sperm in 15ml test tubes has been compared to conventional freezing of 0.025 cc straws (average of accepted range).

In addition, the effect of the thawing and subsequent re-freezing of the sperm has been evaluated.

For the first freezing of the whole ejaculate in 15ml test tube MTG 1600 (IMT LTD) has been employed with the following parameters:

dT = 5C to -50C;

d=10mm; and

V=1mm/sec.

For the second freezing MTG 700 (IMT LTD) has been employed with the following parameters:

dT = 5 to -50;

d= 0.1mm; and

V=1.5mm/sec.

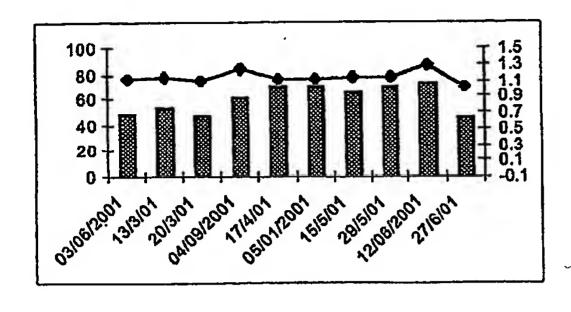
After storage of 1 to 3 weeks, thawing was done by transferring the straws into water bath of 38C. Viability is express as by post thaw motility of the sperm. It is clear that large volume freezing in the MTG apparatus is better to the conventional freezing and it permit second freezing with _ cc straws with overall post thaw survival of 45% which could be use for artificial insemination.

| | volume | motility | SMI* | Concentration | Post thaw |
|-----------------|--------|----------|------|----------------------|-----------|
| | | | | | survival |
| conventional | 4ml | 80% | 77 | 1.2x 10 ⁹ | 55% |
| | | | 77 | 1.2x 10 ⁹ | 70% |
| MTG1600 | 4ml | 80% | / / | 1.22 10 | |
| First freezing | | | | | 45% |
| MTG 700 | | | | | 45% |
| Second freezing | | | CC | | |

The results are summary of three different bulls.

SMI*- Semen Motility Index

Figure 1: Effect of omega-3 on sperm motility (column) and conc. (line) vs time



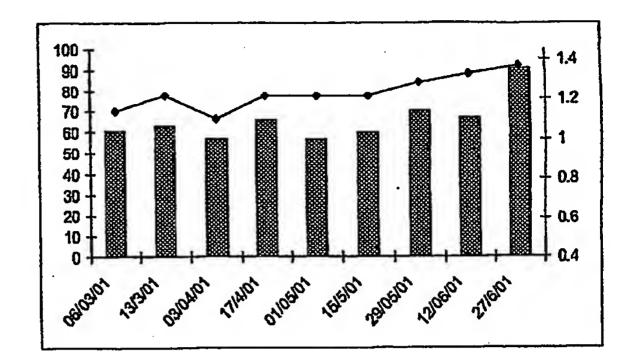
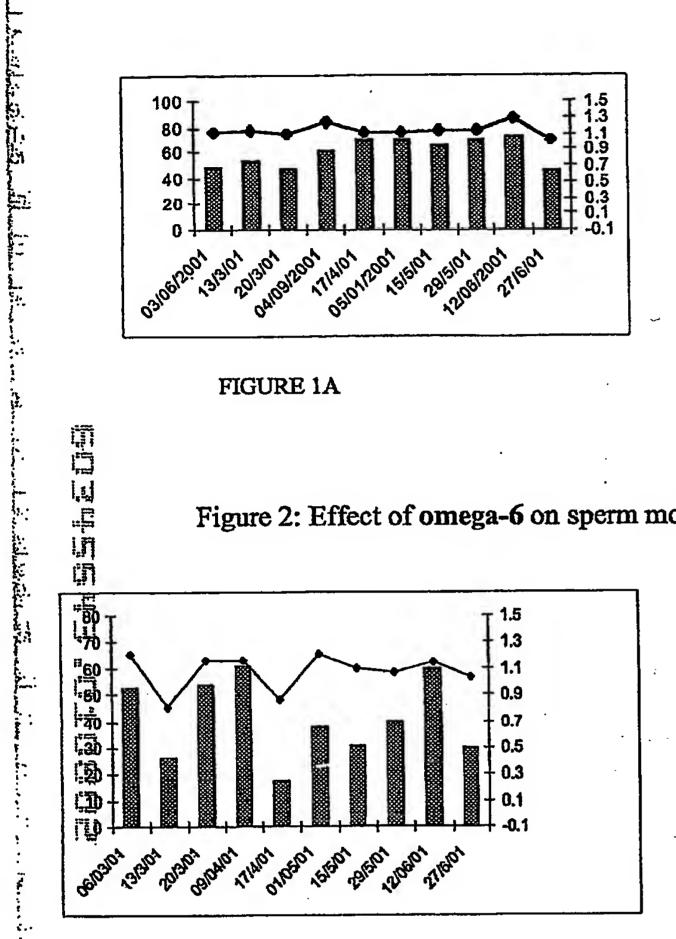


FIGURE 1A

FIGURE 1B

Figure 2: Effect of omega-6 on sperm motility (column) and conc. (line) vs time



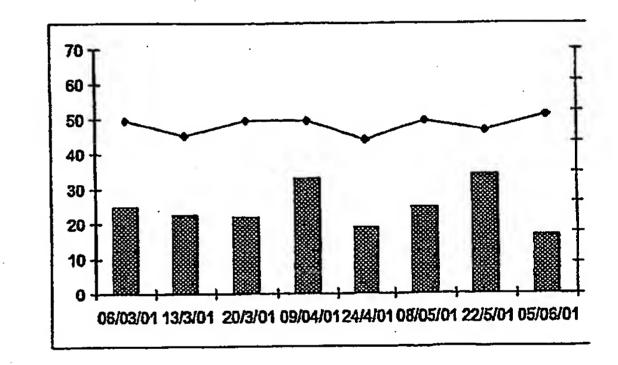
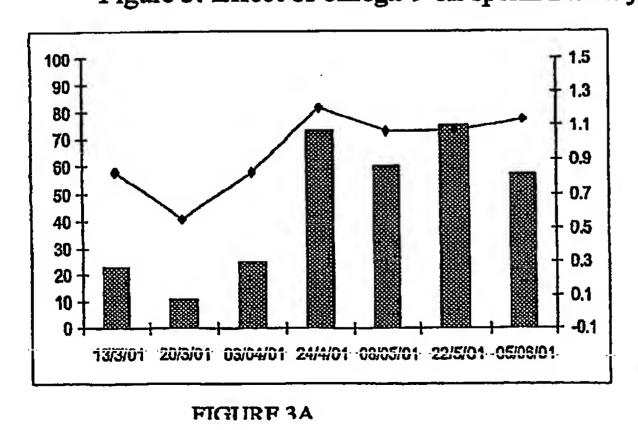
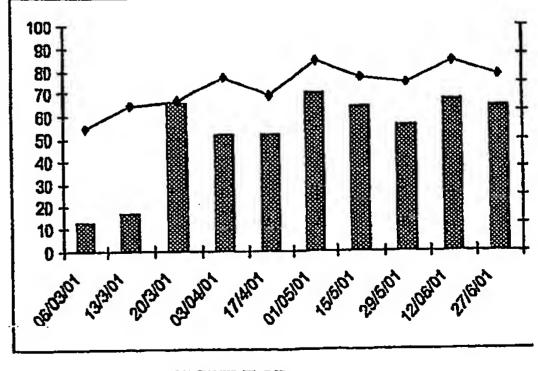


FIGURE 2A

FIGURE 2B

Figure 3: Effect of omega-9 on sperm motility (column) and conc. (line) vs time





FIGURF 3R

What is claimed:

- 1. Dietary supplements for improving at least one semen characteristic in ruminant species essentially as described herein.
- 2. Improved methods for cryopreservation of semen essentially as described herein.

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Application papers not suitable for publication

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